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Rapid, Novel, Specific, High-Throughput Assay for Diagnosis of *Loa loa* Infection▽

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The ability to diagnose *Loa loa* infection readily and accurately remains a demanding task. Among the available diagnostic methods, many are impractical for point-of-care field testing. To investigate whether luciferase immunoprecipitation systems (LIPS) can be used for rapid and specific diagnosis of *L. loa* infection, a LIPS assay was developed based on immunoglobulin G (IgG) and IgG4 subclass antibodies to a recombinant *L. loa* SXP-1 (designated LlSXP-1) antigen and tested with sera from healthy controls or patients with proven infection with *L. loa*, *Mansonella perstans*, *Onchocerca volvulus*, *Strongyloides stercoralis*, or *Wuchereria bancrofti*. A LIPS test measuring IgG antibody against LlSXP-1 readily differentiated *L. loa*-infected from uninfected patients and demonstrated markedly improved sensitivity and specificity compared with an LlSXP-1 IgG4-based enzyme-linked immunosorbent assay (67% sensitivity and 99% specificity). No significant immunoreactivity was observed with *S. stercoralis*-infected sera, but a small number of patients infected with *O. volvulus*, *M. perstans*, or *W. bancrofti* showed positive immunoreactivity. Measuring anti-IgG4-specific antibodies to LlSXP-1 showed a significant correlation ($r \sim 0.85$; $P < 0.00001$) with the anti-IgG results but showed no advantage over measuring the total IgG response alone. In contrast, a rapid LIPS format (called QLIPS) in which the tests are performed in less than 15 minutes under nonequilibrium conditions significantly improved the specificity for cross-reactive *O. volvulus* patient sera (100% sensitivity and 100% specificity). These results suggest that LIPS (and the even more rapid test QLIPS) represents a major advance in the ability to diagnose *L. loa* infection and may have future applications for point-of-care diagnostics.

The development of rapid diagnostic assays for onchocerciasis (22) and lymphatic filariasis (20) has not only simplified the care of individual patients with these filarial infections but also allowed accurate and cost-effective geographic mapping for the purpose of mass chemotherapy in areas of endemicity (21) and for use in the certification process of elimination (15). Nevertheless, the coendemicity of loiasis with these and other filarial infections of humans remains an issue because of species-specific differences in the responses to available antifilarial therapies. This has been of particular concern in the setting of both the African Program for Onchocerciasis Control (APOC) and the Global Program for the Elimination of Lymphatic Filariasis (GPELF), where mass drug administration has ground to a halt in certain areas of Africa because of deaths related to ivermectin administered as part of mass treatment programs for onchocerciasis control (3, 12).

Although the definitive diagnosis of *Loa loa* infection can be made morphologically by identifying microfilariae in the blood or, rarely, after surgical removal of the adult worm (typically from its subconjunctival location), a proportion of infected individuals are amicrofilaremic (9, 13). PCR tests provide highly specific tests for *L. loa* (17–19) but are impractical for field conditions and have not shown significantly improved sensitivity over parasitological methods. Serologic testing by immunoblotting (10) and enzyme-linked immunosorbent assays (ELISAs) (1, 2) with crude, complex mixtures of *L. loa* extracts has shown poor specificity because of cross-reactivity in patients with other filarial infections as well as those with strongyloidiasis. A promising alternative to crude antigen-based immunoassays employs defined, recombinant *L. loa* antigens that show high sensitivity and specificity. One such antigen, LlSXP-1, a member of the Sxp1/λRAL family of nematode proteins, employed in an immunoglobulin G4 (IgG4)-based ELISA, was shown to be a highly specific (>99%) but relatively insensitive (56%) method for diagnosis of *L. loa* infection (14). Despite its low sensitivity, the high predictive value of a positive result in select clinical settings was encouraging. Thus, new methods and/or antigens that can be utilized in diagnosing *L. loa* infection specifically are needed.

We have recently described a highly sensitive immunoprecipitation technology, designated the luciferase immunoprecipitation system (LIPS), that utilizes mammalian cell-produced, recombinant fusion protein antigens for efficiently evaluating antibody responses (4–6). In the present study, LIPS technology was used to develop an assay for *L. loa* infection that is rapid, sensitive, specific, and high throughput. The results presented here demonstrate that LIPS measuring total anti-IgG response against LlSXP-1 produces highly robust values for distinguishing *L. loa*-infected individuals from controls (100% sensitivity and 100% specificity), with only a small degree of cross-reactivity with a few *Onchocerca volvulus*- and

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TABLE 1. Patient populations for serologic studies

Group and source of population[a]	No. of patients
Control	
United States	36
Loiasis (MF^+)	
West/Central Africa	23
Endemic	13
Visitor	10
Loiasis (MF^-)	
West/Central Africa	29
Endemic	2
Visitor	27
Onchocerciasis (MF^+)	
Ecuador	
Endemic	31
West Africa[b]	
Visitor	10
W. bancrofti infection (CFA^+)	
India	36
Strongyloides stercoralis	
South East Asia	31
Loiasis/onchocerciasis coinfection	
West/Central Africa	4
Mansonella perstans	
Mali	10

[a] MF^+, microfilaria positive; MF^-, microfilaria negative; CFA^+, circulating-filarial-antigen positive.
[b] Countries where *L. loa* is not endemic (e.g., Sierra Leone, Liberia).

Wuchereria bancrofti-infected patient sera. A LIPS assay based on detecting anti-IgG4 levels did not improve sensitivity or specificity for determining *L. loa* infection status. In contrast, a rapid LIPS format in which the tests are performed in less than 15 minutes under nonequilibrium conditions significantly improved specificity by likely limiting the opportunity for cross-reactivity of antibodies in *O. volvulus*-infected patient sera. Though not yet point of care, this assay, with distinct positive and negative predictive values, may provide a solution to the problem of quickly and definitively identifying regions of *L. loa* endemicity throughout the world.

MATERIALS AND METHODS

Patient sera. The sera used in this study were from well-characterized patients with loiasis (13), lymphatic filariasis (7), onchocerciasis (11), *Mansonella perstans* infection, or strongyloidiasis (16) or from North American controls participating in NIAID IRB-approved protocols of the Laboratory of Parasitic Diseases, NIAID. The North American controls had no history of exposure to filarial or other nematode parasites, nor had they traveled out of North America. Diagnosis of loiasis was based either on demonstration of microfilariae in the blood ($n = 23$) or, if the subject was microfilaria negative, on extraction of an adult worm ($n = 5$), a positive PCR in the blood ($n = 1$), or positive antifilarial antibodies plus Calabar swellings and response to definitive therapy ($n = 23$). All subjects with lymphatic filariasis were circulating-filarial-antigen positive (20). All subjects with strongyloidiasis were positive on stool examination for *Strongyloides stercoralis* larvae. All subjects with onchocerciasis had demonstrable microfilariae on skin snips; all those with *M. perstans* were microfilaria positive and had had infection with *W. bancrofti* excluded by both night blood filtration and circulating-filarial-antigen testing. A detailed summary of the patient sera used is shown in Table 1. Some of the samples had also previously been analyzed by an LlSXP-1 ELISA, as described elsewhere (14).

Phylogenetic analyses. Using the SXP-1 sequence obtained from *Loa loa* as the query sequence, we performed a BLAST search against the nonredundant nucleotide databases to identify homologues in *O. volvulus*, *W. bancrofti*, and *S. stercoralis*. These sequences were then aligned using the global alignment program ClustalW with the default parameters.

Phylogenetic analysis was performed with PAUP* 4.0b10, using maximum likelihood distances with neighbor-joining clustering and 2,000-bootstrap replicates.

Construction of the LlSXP-1 luciferase construct. pREN2, a mammalian *Renilla* luciferase (Ruc) expression vector, was used to generate all plasmids (5). LlSXP-1 was amplified from an existing plasmid (14) by PCR, using the following gene-specific linker-primer adapter sequences: 5′-GAGGGATCCAATTCGGC ACGAGCAGAA-3′ and 5′-GAGCTCGAGTTATTTTGGACGAAGTGC-3′. Following the PCR, the product was restricted with BamHI and XhoI and ligated into BamHI-XhoI-cut pREN2. The resulting pREN2 expression vector was prepared using a Qiagen midi kit (Qiagen, Gaithersburg, MD). Automated DNA sequencing was used to confirm the integrity of the DNA construct.

LIPS analysis. Extracts containing the Ruc–LlSXP-1 antigen fusions were prepared from transfected Cos1 cells, as previously described (5). Using this Ruc–LlSXP-1 extract, the immunoprecipitation assay was performed with a 96-well plate format at room temperature, essentially as described for other serologic tests (6). Briefly, patient sera were diluted 1:10 in assay buffer A (20 mM Tris, pH 7.5, 150 mM NaCl, 5 mM $MgCl_2$, 1% Triton X-100) in a 96-well polypropylene microtiter plate. For evaluating antibody titers by LIPS, 40 μl of buffer A, 10 μl of diluted human sera (1-μl equivalent), and 50 μl of 1×10^7 luminescence units (LU) of Ruc antigen from the Cos1 cell extract, diluted in buffer A, were added to each well of a second polypropylene plate, which was used to conduct the assay. This plate, containing 100 μl of the antigen-antibody reaction mixture, was then incubated for 1 h at room temperature. Next, 7 μl of a 30% suspension of Ultralink protein A/G beads (Pierce, Rockford, IL) in phosphate-buffered saline was added to the bottom of a 96-well filter high-throughput-screening plate (Millipore, Bedford, MA). The 100-μl antigen-antibody reaction mixture from each microtiter well was then transferred to the well of the filter plate, and this plate was further incubated for 1 h at room temperature on a rotary shaker. The filter plate containing the mixture was then applied to a vacuum manifold. The retained protein A/G beads were washed, and after the final wash, the plate was blotted and LU measured with a Berthold LB 960 Centro microplate luminometer, using a coelenterazine substrate mixture (Promega, Madison, WI). All LU data presented were obtained from the averages for two independent experiments and corrected for background by subtracting LU values of beads incubated with LlSXP-1 Cos1 cell extract but no sera.

For anti-IgG4 antibody determinations, the same protocol was utilized, with anti-IgG4 antibody beads substituted for protein A/G beads. The anti-IgG4 antibody beads were generated by combining 10 mg of an anti-IgG4 monoclonal antibody with Ultralink preactivated beads (Pierce Biotechnology, Boston, MA), as described by the manufacturer. The coupling efficiency was greater than 90%.

QLIPS. A modified, shortened version of LIPS, designated QLIPS (for *q*uick *LIPS*), was also employed with a selected number of sera. In these assays, the patient sera were combined with the LlSXP-1 Cos1 extract and buffer for only 5 min and then incubated for another 5 min with the protein A/G beads. The plate was then washed and read on the luminometer as described above. The total time required to process the samples was less than 15 min.

Statistical analysis. Analysis comparing groups of variables was performed using the Mann-Whitney U test. Correlations were assessed by Spearman rank. All data were analyzed using GraphPad Prism (version 5.0) software.

RESULTS

LIPS detection of anti-LlSXP-1 antibodies for diagnosis of *L. loa* infection. The relationships between LlSXP-1 (GenBank accession no. AAG09181) and the relevant orthologues from *Wuchereria bancrofti* (AAC17637), *Onchocerca volvulus* (P36991), and *Strongyloides stercoralis* (translated from BE579926) are depicted in Fig. 1. As can be seen, LlSXP-1 is quite closely related to SXP-1 from the other filarial species (48% identity and 18% similarity with *W. bancrofti* SXP-1 [Wb–SXP-1] and 43% identity and 17% similarity with *O. volvulus* SXP-1 [Ov–SXP-1]). Despite these similarities, when used as the basis for an IgG-based ELISA (Fig. 2A), bacterially produced recombinant LlSXP-1 showed

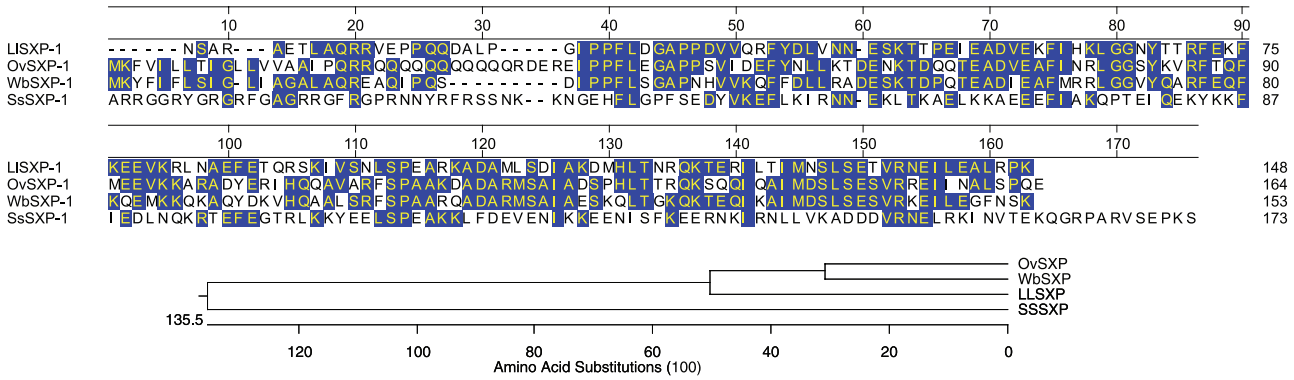

FIG. 1. Phylogenetic analysis and comparison of amino acid sequences of SXP-1 homologues. The relationships between LlSXP-1 from *L. loa* and the relevant orthologues from *Wuchereria bancrofti* (Wb), *Onchocerca volvulus* (Ov), and *Strongyloides stercoralis* (Ss) are shown. Identical amino acids for LlSXP-1 and the other filarial-protein homologues are denoted by the shaded boxes. Below is shown the phylogenetic tree, based on the amino acid differences among the represented species.

reasonable sensitivity (66%) and specificity (88%) when sera from healthy subjects and from patients with closely related filarial infections (*O. volvulus* and *W. bancrofti*) were used. The specificity improved with an IgG4-based approach (Fig. 2B), but with significant loss of sensitivity (Table 2), as has been previously reported (14).

A full-length LlSXP-1 cDNA was constructed as a fusion protein with Ruc. Transfection of 100-mm^2 dishes of Cos1 cells with this Ruc–LlSXP-1 fusion construct typically yielded high expression levels of the fusion protein, producing over 3×10^9 LU per plate. By use of the Cos1 cell-produced Ruc–LlSXP-1 extract, blinded sera were tested at two separate times in the LIPS format. The geometric mean for two independent immunoprecipitation tests for LlSXP-1 was obtained with values showing a wide dynamic range, from 0 to 2.7 million LU, in the samples. The 36 healthy-control samples had very low signals, with a geometric mean anti-SXP-1 antibody titer of 1.25 LU (Fig. 3A). For the 17 *L. loa* patients, the geometric mean value was close to 10^6 times higher, at 1.4×10^6 LU, which was highly statistically significantly different ($P < 0.0001$) from that for the healthy, uninfected patients. A similar distinction could

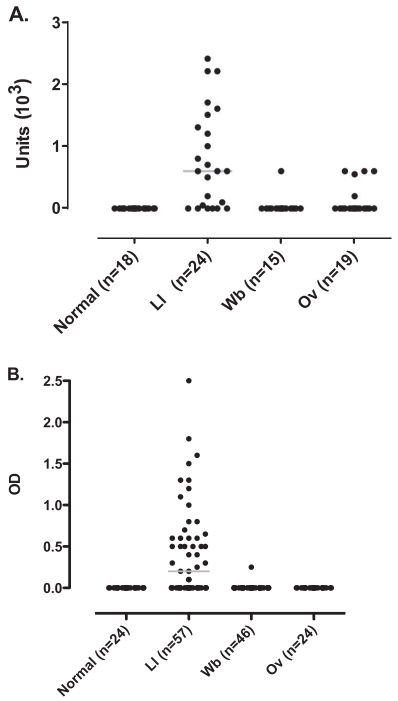

FIG. 2. ELISA detection of anti-SXP-1 IgG and IgG4 antibody titers in healthy controls and patients infected with *L. Loa* (LI), *W. bancrofti* (Wb), and *O. volvulus* (Ov). Each symbol represents the geometric mean for an individual serum sample run in duplicate for IgG (A) and IgG4 (B) anti-LlSXP-1, with the horizontal gray bars representing the medians for the groups. OD, optical density.

TABLE 2. Sensitivity, specificity, and positive and negative predictive values for the various *L. loa*-specific antibody-based assays[a]

Assay type	Comparison	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
ELISA					
IgG	*L. loa* vs no filariae	67	100	100	67
	L. loa vs other filariae	67	81	72	77
IgG4	*L. loa* vs no filariae	47	100	100	49
	L. loa vs other filariae	47	99	96	73
LIPS					
IgG	*L. loa* vs no filariae/*S. stercoralis*	100	100	100	100
	L. loa vs other filariae	94	78	48	98
IgG4	*L. loa* vs no filariae/*S. stercoralis*	94	99	94	99
	L. loa vs other filariae	93	81	48	98
QLIPS					
IgG	*L. loa* vs other filariae	97	100	94	100

[a] PPV, positive predictive value; NPV, negative predictive value.

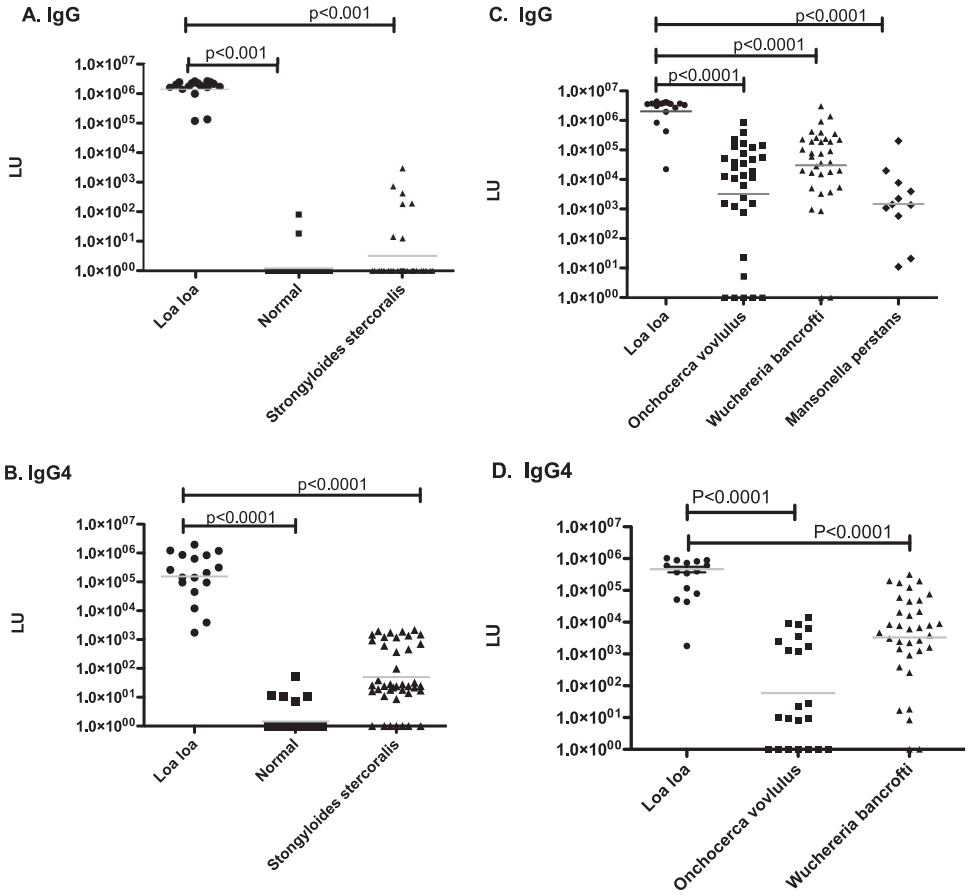

FIG. 3. Distribution of anti-LlSXP-1 IgG and IgG4 antibody levels as determined by LIPS in healthy controls, *L. loa*- and *S. stercoralis*-infected patients (A and B), and *L. loa*-, *W. bancrofti*-, *O. volvulus*-, and *M. perstans*-infected patients (C and D). The anti-LlSXP-1 IgG (A) and IgG4 (B) antibody titers are shown for sera from healthy individuals and those with *L. loa* and *S. stercoralis*. The anti-LlSXP-1 IgG (C) and IgG4 (D) titers in additional sera from patients with *L. loa*, *O. volvulus*, *W. bancrofti*, and *M. perstans* infection are shown. Each dot represents the geometric mean number of LU in an individual patient's serum sample run in duplicate, and the horizontal gray bars represent the geometric mean for each diagnostic group.

be made between the *L. loa*-infected patients and those with proven *S. stercoralis* infection. With a cutoff of 5,000 LU for the anti-SXP-1 antibody titer, LIPS showed 100% sensitivity and 100% specificity in this analysis. For LIPS, none of the 17 *L. loa*-infected patient sera fell below an arbitrary cutoff value of 10,000 LU. Thus, in this preliminary analysis, IgG LIPS showed a markedly higher sensitivity (Table 2) than the 66% sensitivity observed with IgG ELISA. When the same LIPS assay was used to measure IgG4-specific antibodies to LlSXP-1, the assay performed as well as the IgG-based assay, but the difference between control sera and those from patients with *L. loa* infection was less dramatic (Fig. 3B).

LIPS can distinguish *L. loa* infection from other filarial infections. Sera from patients with filarial infections often have broad filarial antigen cross-reactivity (3, 4). Because of the close phylogenetic relationships among the filarial species (Fig. 1) (14) and because *L. loa* infection often occurs coincidently in areas where *W. bancrofti*, *O. volvulus*, and *M. perstans* are endemic, additional sera from *W. bancrofti*-, *O. volvulus*-, *M. perstans*-, and *L. loa*-infected patients were tested. As seen in Fig. 3C, the geometric mean value for the *L. loa*-infected patients was 2×10^6 LU, whereas the geometric mean value for *O. volvulus*-infected patients was 3,209 LU, that for the *W. bancrofti*-infected patients was 30,478 LU, and that for the *M. perstans*-infected patients was 1,487 LU. Comparison of antibody levels between the *L. loa*-infected patients and those with other filarial infections revealed that they were markedly different ($P < 0.0001$ for *O. volvulus*, *W. bancrofti*, and *M. perstans*). If one utilizes the upper 99% confidence interval of the geometric mean values for the other filarial infections (141,576 LU) as a cutoff, then the assays show a sensitivity of 94% and a specificity of 77% (Table 2), based on 15/16 *L. loa* sera being positive and 17/76 sera from *O. volvulus*, *W. bancrofti*, and *M. perstans* being positive. These results suggest that the LIPS LlSXP-1 test for diagnosing *L. loa* infection showed limited cross-reactivity with other filarial infections. For IgG4 anti-LlSXP-1, the assay performed similarly (Fig. 3D), with 14/15 *L. loa*-infected patients and 9/47 non-*L. loa* filaria-infected patients being positive and with a strong relationship between IgG and IgG4 anti-LlSXP-1 antibody levels ($P < 0.00001$; $r = 0.892$).

Examining a second cohort of *O. volvulus*- and *L. loa*-infected and *L. loa*/*O. volvulus*-coinfected sera by LIPS and QLIPS. Because of the clinical importance of distinguishing *L.*

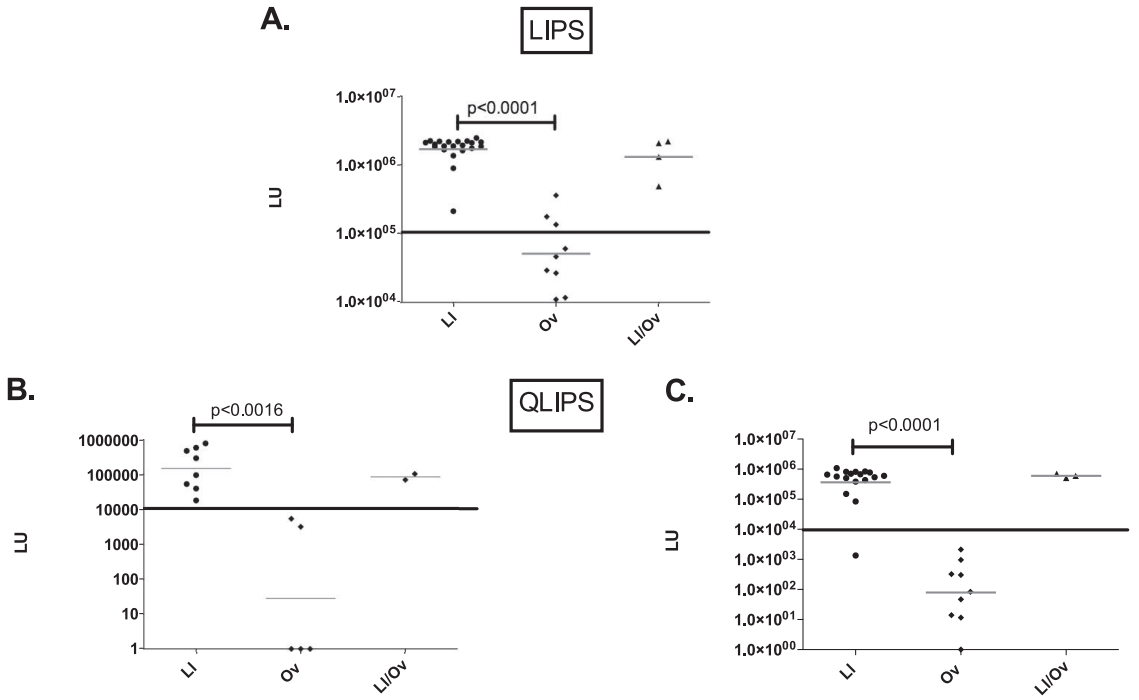

FIG. 4. QLIPS format improves diagnostic specificity. Anti-LlSXP-1 antibody titers determined by LIPS (A) and QLIPS (B and C) in patients with *L. loa* (Ll) and *O. volvulus* (Ov) infection and *L. loa-O. volvulus* coinfection (Ll/Ov). Each dot represents the geometric mean number of LU in an individual patient's serum sample, and the horizontal bars represent the geometric mean for each diagnostic category. The horizontal dark line represents the cutoff between positive and negative samples in each assay.

loa infection from onchocerciasis—as the standard drug therapy (diethylcarbamazine) for *L. loa* is absolutely contraindicated for *O. volvulus* infection and ivermectin (the standard therapy for onchocerciasis) has been associated with severe, life-threatening adverse reactions in patients with loiasis (3, 12)—a second, independent set of sera, composed of sera from 9 patients with *O. volvulus* infection, 21 with *L. loa* infection, and 4 with *L. loa* and *O. volvulus* coinfection, was also evaluated using the IgG LlSXP-1-based LIPS assay (Fig. 4A). From the analysis of the sera in this cohort, 21/21 (100%) of the *L. loa* sera were positive with a cutoff of 100,000 LU, as were 4/4 *L. loa-O. volvulus*-coinfected sera. However, three of the nine *O. volvulus*-infected sera (67%) were cross-reactive and positive by LIPS (Fig. 4A). These results confirm that there are false-positive reactions among the non-*L. loa* filarial infections.

Studies with human immunodeficiency virus- and hepatitis C virus-infected sera have shown that accurate determination of antibody titers, and thus infection status, can be performed with single-tube assays in a quick, 2-min "QLIPS" LIPS format (P. D. Burbelo, unpublished data). Moreover, this format favors detection of antibodies with higher affinity for a given antigen. Thus, we tested whether the LlSXP-1 antigen could be employed in a QLIPS format and whether such a format is able to discriminate better between *L. loa* infection and other filarial infections. In these experiments, 16 preselected sera (the *L. loa*-infected sera with the lowest IgG anti-LlSXP-1 LU values and the *O. volvulus*-infected sera with the highest IgG anti-LlSXP-1 LU values) were tested in a 15-min QLIPS format. As shown in Fig. 4B, a dynamic range of anti-LlSXP-1 antibody titers was observed in QLIPS, ranging from 0 to 780,000 LU. With a cutoff of 10,000 LU, eight of the eight *L. loa* sera were positive, and none of the six *O. volvulus*-infected sera were positive. As would have been predicted, two of the two *O. volvulus*/*L. loa*-coinfected individuals were also positive. In a second set of serum samples, the QLIPS results were largely similar, with all but 1 of 21 *L. loa* sera being positive and all 9 of the sera from *O. volvulus*-infected sera being negative (Fig. 4C). These results suggest that this quick, 15-min LIPS format shows extraordinary sensitivity and specificity (Table 2) for the diagnosis of *L. loa*-infected individuals.

DISCUSSION

This study demonstrates that LIPS can be used to identify *L. loa*-specific antibody levels and has utility for the diagnosis of *L. loa* infection. Compared to the previously described LlSXP-1 ELISA that had relatively poor sensitivity (55%) despite high specificity (14), a LIPS-based assay using the same antigen showed increased sensitivity without a loss of specificity. This increased sensitivity in the anti-LlSXP-1 antibody test is likely due to the ability of LIPS, which is performed with solution, to detect many more conformational epitopes than a standard solid-phase ELISA. Compared with the current ELISA, LIPS can identify *L. loa*-infected patients more rapidly and with greater accuracy. In addition to 96% sensitivity and 100% specificity, a robust difference in antibody titers was observed between positive *L. loa*-infected sera and the negative control sera. While not studied in detail here, the extraordinary dynamic range of antibody titers observed in *L. loa*-infected sera might have additional utility for understanding

individual immune responsiveness, severity, and duration of infection and response profiles of drug therapy.

The antigen used in LIPS was the LlSXP-1 protein. Previous analysis revealed that homologues of the SXP-1/Ral family of nematode proteins include Ov17 of *O. volvulus*, Wb–SXP-1 of *W. bancrofti*, and Bm–SXP-1 of *Brugia malayi* (14). At the primary amino acid levels, the best homology of LlSXP-1 is with its homologues in *Brugia malayi*, *W. bancrofti*, and *O. volvulus*. The similarities among these proteins likely account for the cross-reactive antibodies (given sufficient incubation times) and imperfect sensitivity of an SXP-based test. Nevertheless, there were substantial differences in antibody levels among the different filaria-infected sera tested. The marked increase in sensitivity and the robust nature of LIPS are likely related to the solution phase reaction that may allow detection of many conformational epitopes that are missed by the solid-phase ELISA arrays. Interestingly, none of the *S. stercoralis*-infected sera showed cross-reactivity, which is consistent with the *S. stercoralis* SXP being quite distinct phylogenetically (Fig. 1) from the filarial SXPs. Unlike for the ELISA studies, in which monitoring anti-IgG4 antibody levels in filarial infections can increase specificity (8, 14), no increase in sensitivity or specificity was found by measuring anti-IgG4 levels compared with total IgG levels in the LIPS format. Thus, the standard LIPS format employing protein A/G beads represents a less expensive and simple format for detecting these antibodies.

Compared with the standard 2.5-h LIPS test, which includes two sequential 1-h incubations, the QLIPS format showed promising results. In this 15-min QLIPS format, all of the *L. loa*-infected patient sera showed robust signals, while many of the *O. volvulus*-infected sera that were previously false positive in the standard 2.5-h format were now negative. A likely explanation is that *O. volvulus*-infected sera contain antibodies that are of much lower affinity for LlSXP-1 antigen and thus fail to bind as readily during this short incubation period, resulting in much lower signals for these sera. An even faster version of this assay can be performed in less than 2 min on a few samples by eliminating the incubation steps (P. D. Burbelo, unpublished data). In addition, finger-stick blood draws containing contaminating red blood cells and other components do not interfere with the LIPS assay and thus eliminate the expense and expertise associated with phlebotomy (P. D. Burbelo, unpublished data). Further studies are needed to explore the usefulness and accuracy of this format using finger-stick blood draws, with the objective of establishing an efficient point-of-care test. It should be noted that there are portable, battery-operated luminometers that could be utilized under field conditions.

While there are many reports demonstrating diagnostic tests for filarial infections, the diagnostic specificity and sensitivity of many of these tests are not optimal, and thus, treatment cannot be effectively applied. In addition to *L. loa* infection, LIPS has been used to accurately diagnose and monitor treatment for *S. stercoralis* infection (16). In these studies, the use of two different *S. stercoralis* antigens dramatically improved the diagnostic sensitivity over that observed for a single antigen alone. It is likely that the incorporation of other *L. loa*-specific antigens would increase the sensitivity and specificity as well as the diagnostic utility of these LIPS serological tests. An application of LIPS with a comprehensive panel of antigens for different filarial infections, including those caused by *L. loa*, *O. volvulus*, *W. bancrofti*, and *M. perstans*, can be incorporated into this system so that the high discriminative power of LIPS can be applied for accurate diagnosis and monitoring of appropriate treatment.

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